

# Genie<sup>®</sup> II

## USER MANUAL

OptiGene Ltd.  
Unit 5, Blatchford Road,  
Horsham,  
West Sussex,  
RH13 5QR

Tel: +44 (0) 1403-274980

Fax: +44 (0) 1403-271017

[www.optigene.co.uk](http://www.optigene.co.uk)

[info@optigene.co.uk](mailto:info@optigene.co.uk)

Information in this document is subject to change without notice.

OptiGene Ltd. assumes no responsibility for any errors that may appear in this document. This document is believed to be complete and accurate at the time of publication.

In no event shall OptiGene Ltd. be liable for incidental, multiple, or consequential damages in connection with or arising from use of this document.

Genie® II and its design is a registered trademark of OptiGene Ltd. or its subsidiaries in the UK and other countries.

---

# Table of Contents

<b>TABLE OF CONTENTS</b> .....	I	<b>RUN</b> .....	16
<b>SAFETY NOTICES</b> .....	3	profile <b>SCREEN</b> .....	16
<b>SUPPORT</b> .....	5	To create a new profile.....	16
How to obtain support.....	5	To load a saved profile .....	17
Supported consumables .....	5	<b>ACTIVE</b> .....	20
<b>BOX CONTENTS</b> .....	6	Profile .....	20
<b>SITE PREPARATION</b> .....	7	Temperature.....	20
How to set up Genie® II .....	7	Amplification .....	21
<b>CONNECTIONS</b> .....	8	Anneal.....	22
<b>OPENING AND CLOSING THE LID</b> .....	8	Results .....	22
<b>INSERTING TUBES</b> .....	9	<b>A/B</b> .....	22
Battery .....	9	<b>NOTES</b> .....	23
Battery monitor .....	10	<b>VIEW</b> .....	23
Screen Brightness Control .....	11	<b>CONNECTING TO A COMPUTER</b> .....	27
<b>EMBEDDED SOFTWARE</b> .....	12	Genie® II Software updates .....	31
Genie® II Welcome Screen.....	12	Via PC.....	32
Main Menu .....	13	Via Instrument (v1.17 & Later) ....	34
Initial set up .....	13	<b>PC SOFTWARE</b> .....	36
Utilities.....	14	Welcome Screen.....	36
Touchscreen calibration .....	14	Quick start buttons.....	36
Set Time and Date.....	15	Setting up a run .....	37
Turn off .....	15	Profile Screen.....	37
		Notes .....	37
		Assays .....	38
		Wells .....	38
		Profile .....	38
		Graphs .....	40
		Peak Classification .....	40
		Genotype .....	41
		Run.....	41

---

Observing a run.....	42
Profile.....	42
Isothermal.....	42
Isothermal Ratio .....	42
Anneal .....	43
Anneal Derivative.....	43
Peaks.....	44
Results.....	44
 Analysis and Graph Options.....	45
Genie® II Graph Options .....	45
 Opening previous run files.....	47
 Additional features .....	47
 TECHNICAL SPECIFICATION.....	50

## SAFETY NOTICES

Please read the following notices carefully before using Genie® II.

The equipment supplied has been designed to consider the normal likely operation and features have been designed in to avoid risks associated with this use. However to avoid any risk to the safety of the equipment, operator, or those in the same area as the equipment, please read this chapter with care before unpacking and using the instrument. If there is any doubt as to the correct use of the equipment please contact the vendor.

### Notices



Using the instrument in a manner not specified by OptiGene Ltd. may result in personal injury or damage to the instrument.



Always ensure that the surface on which the instrument is placed is level and stable and will not cause the instrument to topple over. Ensure the surface is suitable for the weight and size of the instrument. If the instrument is dropped it may cause harm.



The instrument should never be lifted by its covers. Always ensure that the base or sides are used as the lifting point.



The instrument is electrically powered. Please ensure that the correct voltage settings have been applied before applying power to the instrument. If in doubt consult a qualified electrician. The instrument has a rating label affixed to the rear. Please consult this if needed.



Always disconnect the equipment before moving or removing any guards or covers. Switch it off at the mains outlet, remove the mains plug from the wall socket and remove the cable from the inlet socket on the rear.



While every effort has been made to protect the inside of the instrument against splashes, the instrument carries no IP rating. If fluids are spilt over the instrument they may get inside and cause a dangerous situation with respect to the voltage within the enclosure.



If a spill occurs that may pose a danger, remove power from the instrument. Do not touch the instrument or any fluid flowing from it while it is connected to the live mains supply. Always follow local health and safety guidelines.

Normal safe local operating standards should be applied in general terms. The items above are for guidance and are not a definitive list.

Please consult the instrument supplier if there is any doubt.

## Disconnection Method



Genie® II is disconnected by removal of incoming mains power source to the unit. Following disconnection the unit should be left for a period of at least 5 minutes before any internal assemblies are removed or examined.



When in use the heating blocks and heated lid are hot, please allow to cool before touching the surfaces.



Safe removal of fluids from Genie® II will depend on the chemistry used. This will also require knowledge of the fluids used in the system to adhere with local health and safety and COSHH regulations. If in doubt, consult the person responsible for the equipment in the laboratory.

# Chapter

# 2

## SUPPORT

### HOW TO OBTAIN SUPPORT

For the latest services and support information go to <http://www.optigene.co.uk/support.htm>

**IMPORTANT!** When directed to do so, contact OptiGene Ltd. to schedule maintenance or calibration of a Genie® II instrument.

### SUPPORTED CONSUMABLES

**IMPORTANT!** Genie® II uses a proprietary tube strip that maximise optical and thermal efficiencies. **Other tubes and strips will not fit.**

**IMPORTANT!** Forcing non supported consumables will cause damage to the instrument and invalidate warranty.

**IMPORTANT!** The shape of the tubes is such that they will only fit in one orientation. The locating pins on the block have corresponding holes in the strips.

Catalogue number	Description
ISO-001	Isothermal Master Mix
OP-0008-50	Genie® II tubes pack size 50 strips
OP-0008-500	Genie® II tubes pack size 500 strips

## BOX CONTENTS

The following is a list of contents in the box for Genie® II:

- Genie® II instrument
- Power supply
- Power lead
- USB connection lead
- USB memory stick containing Genie® II software and this manual as a '.PDF' file
- 20 x Genie® II tubes (optional)
- Sample preparation blocks (optional)



## SITE PREPARATION

### HOW TO SET UP GENIE® II

The laboratory bench should be level and stable. The instrument should be placed centrally on the lab bench and the surfaces surrounding the instrument must be clear of obstructions at all times.

Care must be taken not to unduly restrict the air inlet at the rear of the instrument and the outlet vent at the front of the instrument must not be covered. Restricting airflow by placing objects in front of these areas will impede operation and significantly affect performance.

Electrical points should be close to the instrument to avoid injury from trailing wires.

It is recommended that the instrument is kept away from sinks and other wet areas. Genie® II is an electrical instrument and care should be taken not to operate if there is a risk of water damage.

## CONNECTIONS

Genie® II is ready to use straight out of the box without any connections as it can be used as a standalone instrument and has an internal battery. However to charge the battery or to use Genie® II with a computer, some connections must be made.

Connect the power supply plug into the back of the instrument and then attach the power cable to the supply.

Located to the rear of the instrument is an on/off power button, switch to on and Genie® II will power up and progress through its checks.



## OPENING AND CLOSING THE LID

To open and close the instrument lid(s) in order to add or remove samples, gently lift the lid and it should open upwards. Close the lid by lowering gently.



Care must be taken to ensure that objects are not obstructing the lid when trying to close it and under no circumstances should the lids be forced open or closed.

## INSERTING TUBES

Each of the heating blocks has locating pins.

Genie® II strips have holes that locate on these pins. The strips will only fit in one orientation.

**IMPORTANT!** Genie® II uses a proprietary tube strip that maximises optical and thermal efficiencies. Other tubes and strips will not fit.

## BATTERY

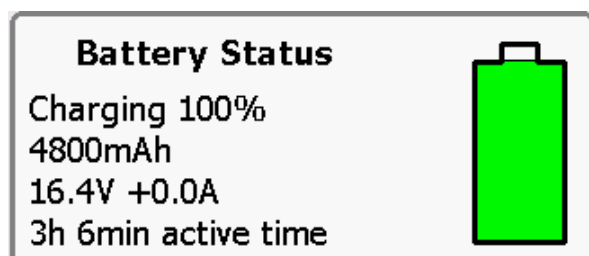
Genie® II has an internal rechargeable battery. When Genie® II is delivered the battery should be fully charged and ready for use.

The battery monitor is on the status bar next to the date and time.



To see more details on the battery, press the battery icon and the monitor will appear as a pop-up in the bottom right hand corner of the screen.

To remove the pop-up press on the status bar indicator again.



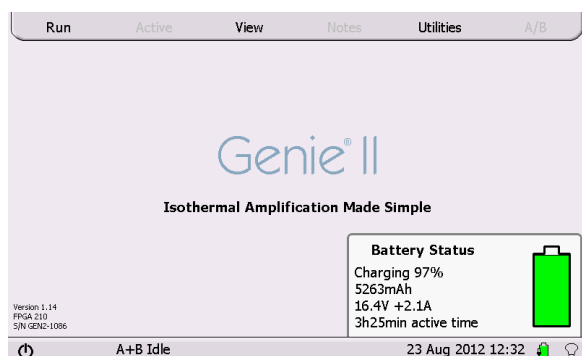
Battery monitor pop-up

**IMPORTANT!** Genie® II's internal battery will only charge when the instrument is plugged into mains electricity and the instrument is switched on. Genie® II can then be shut down using the 'Turn Off' button within the 'Utilities' menu. The LED above the display will glow brightly until the battery is fully charged. At this point Genie® II can be switched off using the switch on the rear.

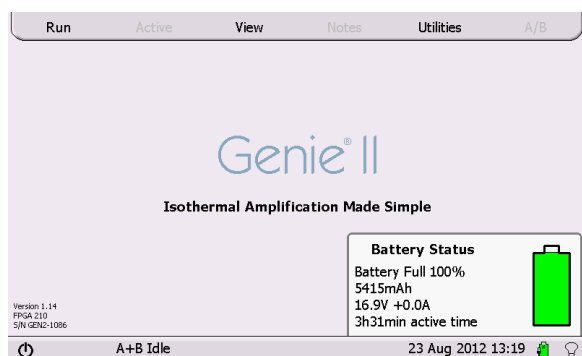
# BATTERY MONITOR



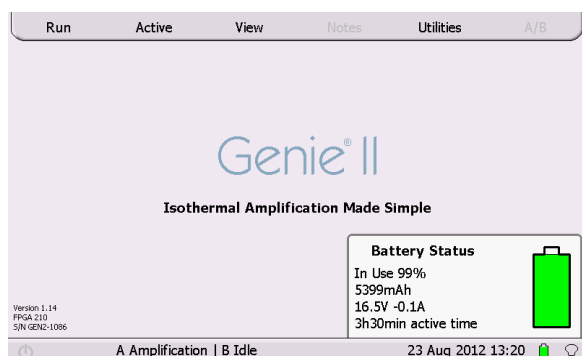
The battery status can be seen but there is no pop-up.



Here the pop-up shows that the battery is currently charging and is at 97%.



Here the pop-up shows that the battery is fully charged.



The pop-up shows that the instrument is in use and the battery is at 99%.

# SCREEN BRIGHTNESS CONTROL

Next to the battery icon is the brightness control.



To utilise, touch the icon and a slider will appear on the right hand side of the screen. Move the slider to the desired position. Press the icon again to remove the slider.



## EMBEDDED SOFTWARE

Genie® II uses a touchscreen for viewing and inputting data.

Touch the screen gently and press the appropriate keys when required.

***IMPORTANT!*** Do not use a pen or any other sharp implements on the screen otherwise damage could occur.

### GENIE® II WELCOME SCREEN

When switching on, the LED above the screen will be **amber** in colour, wait for the light to change to **green**, then touch the screen to access the main menu.



## MAIN MENU



To begin a new run click 'Run'.

To view profiles or previous runs click 'View'.

To access the utilities click 'Utilities'.

## INITIAL SET UP

*When running for the first time check the time and date on the lower status bar.*

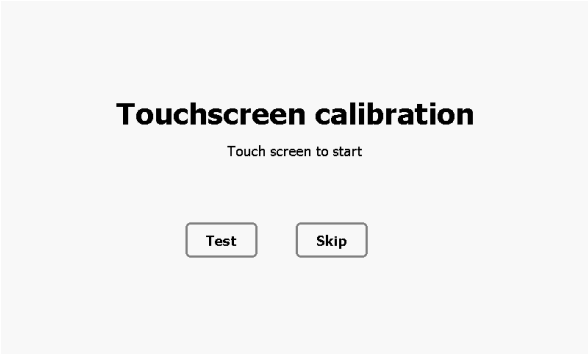


*If either are incorrect then proceed to change the time and date.*

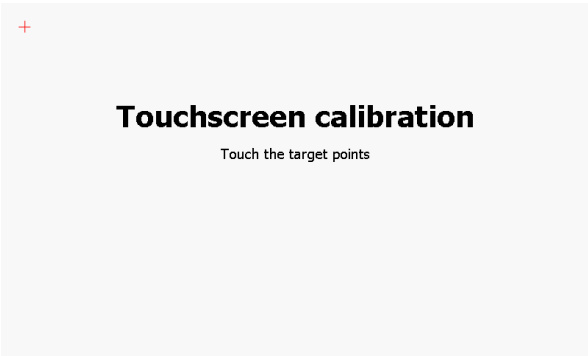
*The time and date settings are in the 'Utilities' menu.*

# UTILITIES

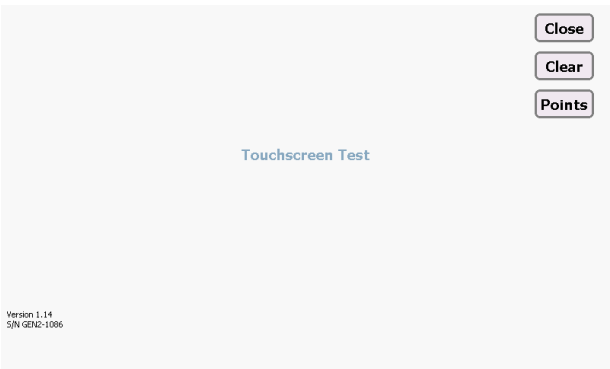
## TOUCHSCREEN CALIBRATION



Touching anywhere on the screen with the exception of Test or Skip will invoke the touchscreen calibration



Here the user is required to touch the target points shown on the screen, this will calibrate the touchscreen



To check the sensitivity of the screen press 'Test'.  
Any point now pressed on the screen will be displayed.



## SET TIME AND DATE

Set Date and Time

Date

Time


7	8	9
4	5	6
1	2	3
0	/	:

⏻ A+B Idle 23 Aug 2012 14:16 🔋💡

Click in the white box for date and enter the date. The date should be in the format DD/MM/YY.

Click in the white box to enter the time. The format should be HH:MM:SS

## TURN OFF

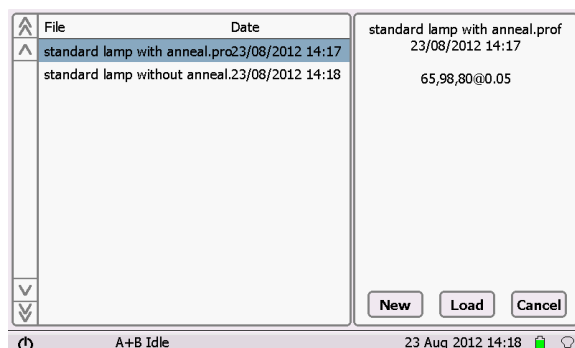
Pressing 'Turn Off' will place Genie® II into standby. Genie® II can also be placed into standby mode by pressing and holding the  symbol on the lower status bar.

Note: If this button is pressed during a run, Genie® II will not enter standby.

Normal operation can be resumed by pressing anywhere on the screen.

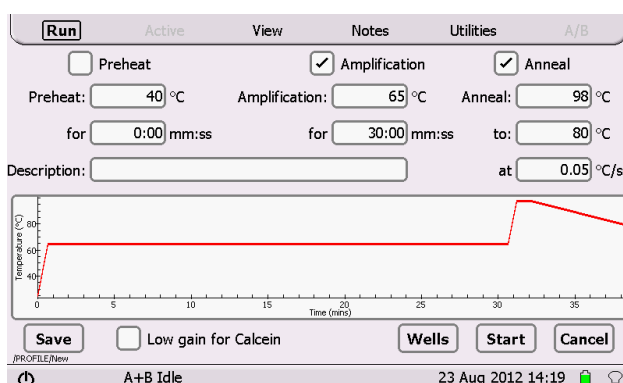
# RUN

## PROFILE SCREEN

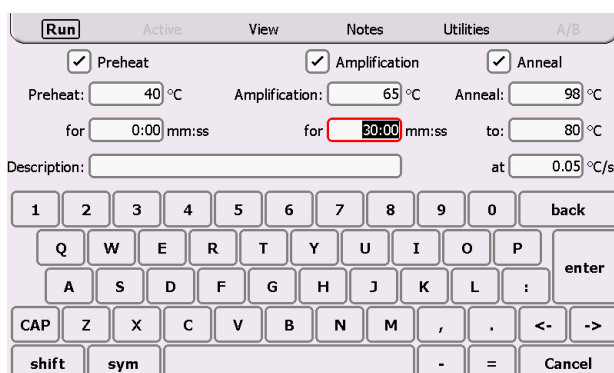


Pressing 'Run' will take the user to the profile screen. Here the user can choose to create a new profile, load an existing profile or cancel and return to the main menu.

## TO CREATE A NEW PROFILE



Press the 'New' button on the profile screen. Adjust the profile by touching the appropriate temperature or time box. Inputs are made using the onscreen keyboard, which will appear as a pop-up, as in the example below.



Once the required changes have been made, pressing 'Enter' will cause the onscreen keyboard to disappear and the profile can now be saved.

Clicking 'Save' will save the profile. Name the profile, press 'Okay' and it will be saved within the 'Profiles' directory on the on-board memory allowing it to be loaded for future runs.

To set a gradient across a block, enter a range of temperatures in the 'Amplification' temperature box. The range of temperatures should be entered separated by a hyphen, as shown below.

## TO LOAD A SAVED PROFILE

From the profile screen choose the profile to be loaded and press the 'Load' key.

The profile will be loaded and details will be displayed on the next screen.

**Run** Active View Notes Utilities A/B

☐ Preheat ☒ Amplification ☒ Anneal

Preheat: 40 °C Amplification: 65 °C Anneal: 98 °C

for 0:00 mm:ss for 30:00 mm:ss to: 80 °C

Description: at 0.05 °C/s

Save ☐ Low gain for Calcein Wells Start Cancel

/PROFILE/New

A+B Idle 23 Aug 2012 14:19

Alterations can be made if desired. To begin the run press 'Start'.

**Run** Active View Notes Utilities A/B

☐ Preheat ☒ Amplification ☒ Anneal

Preheat: 40 °C Amplification: 65 °C Anneal: 98 °C

for 0:00 mm:ss for 30:00 mm:ss to: 80 °C

Description: at 0.05 °C/s

Save ☐ Low gain for Calcein Wells Start Cancel

/PROFILE/standard lamp with anneal.prof

A+B Idle 23 Aug 2012 14:25

**Start Run**

A B Both Cancel

A prompt will ask whether the experiment is to be run on block A, block B, or both.

## CALCEIN DETECTION

This option is if the user is running experiments with Calcein rather than Fluorescein.

☒ Low gain for Calcein

Click on the tick box when using Calcein.

## WELL NAMES

The 'Run' screen displays various settings for a PCR run. At the top, there are tabs for 'Run', 'Active', 'View', 'Notes', 'Utilities', and 'A/B'. Below these are checkboxes for 'Preheat', 'Amplification', and 'Anneal'. The 'Amplification' and 'Anneal' checkboxes are checked. The 'Preheat' is set to 40 °C, 'Amplification' to 65 °C, and 'Anneal' to 98 °C. There are also fields for time (0:00 mm:ss for preheat, 30:00 mm:ss for amplification) and a rate (80 °C to, 0.05 °C/s). A 'Description' field is empty. Below the settings is a graph showing temperature (°C) on the y-axis (40 to 80) versus time (min) on the x-axis (0 to 35). The graph shows a red line that starts at 40 °C, rises to 65 °C, stays there for 30 minutes, and then rises to 98 °C. At the bottom, there are buttons for 'Save', 'Low gain for Calcein', 'Wells', 'Start', and 'Cancel'. A red arrow points to the 'Wells' button. The status bar at the bottom shows 'A+B Idle' and the date/time '23 Aug 2012 14:19'.

To assign names to the wells, click on the 'Wells' button.

The 'First Block well names & abbreviations' screen shows a table with two columns: 'Well Name' and 'Abbreviation'. There are 8 rows. The first two rows are pre-filled with 'Sample 1' and 'Sample 2'. The third row has 'Sampl' in the 'Well Name' field. Below the table is a numeric keypad (1-0) and a 'back' button. Below that is a QWERTY keyboard layout. At the bottom are buttons for 'CAP', 'Z', 'X', 'C', 'V', 'B', 'N', 'M', ',', '.', '<-', and '>-', along with 'shift', 'sym', '-', and '=' buttons. On the right side of the screen are buttons for 'More', 'Okay', and 'Cancel'.

To change a well name, press on the well and type a name.

'More' switches to the second block

'Okay' returns back to the run screen with changes made.

'Cancel' returns to the run screen with no changes made.

The 'Second Block well names & abbreviations' screen shows a table with two columns: 'Well Name' and 'Abbreviation'. There are 8 rows. The first four rows are pre-filled with 'Well 9', 'Well 10', 'Well 11', and 'Well 12'. The next four rows are pre-filled with 'Well 13', 'Well 14', 'Well 15', and 'Well 16'. Below the table is a numeric keypad (1-0) and a 'back' button. Below that is a QWERTY keyboard layout. At the bottom are buttons for 'CAP', 'Z', 'X', 'C', 'V', 'B', 'N', 'M', ',', '.', '<-', and '>-', along with 'shift', 'sym', '-', and '=' buttons. On the right side of the screen are buttons for 'More', 'Okay', and 'Cancel'.

Second block well names.

If the profile is saved at this point the well names and abbreviations will also be saved as part of the profile.

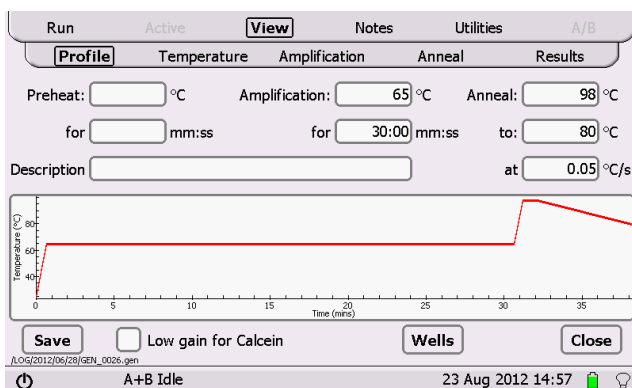
The well names screen can be accessed at any time the instrument is running by returning to the profile screen and pressing the 'Wells' button.

# ACTIVE

Once the run has started the 'Active' screens (which are no longer greyed out) will be accessible.

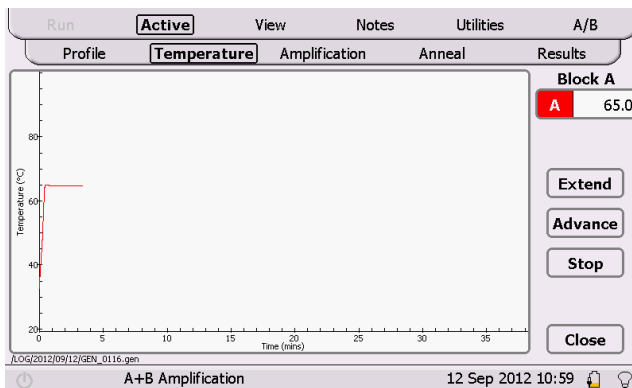


The software will automatically go to the 'Temperature' screen initially. The other screens can be accessed using the tabs below 'Active'.



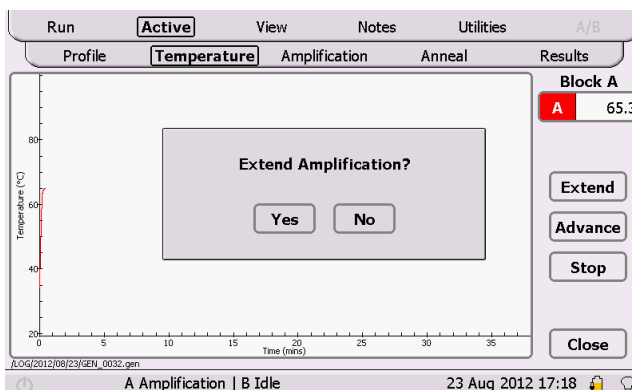
## PROFILE

This shows the profile that is running.



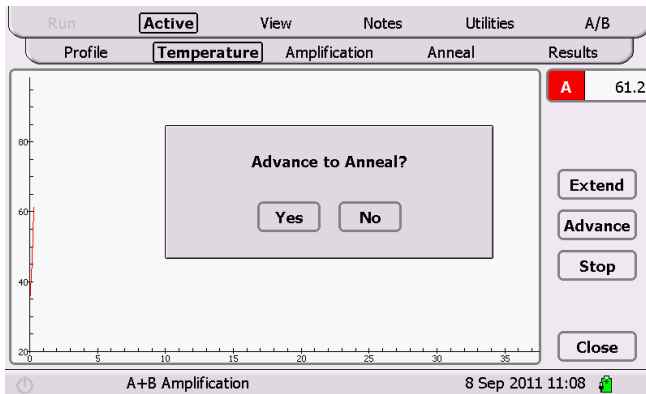
## TEMPERATURE

This shows the temperature of the block(s) as the experiment is progressing.



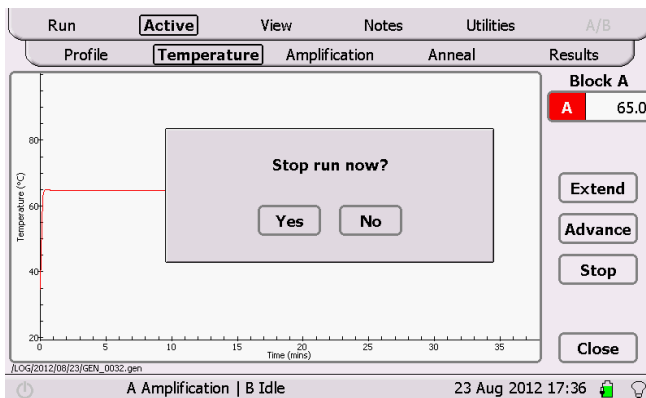
## EXTEND

This allows 10 minutes to be added to the current phase of the run.



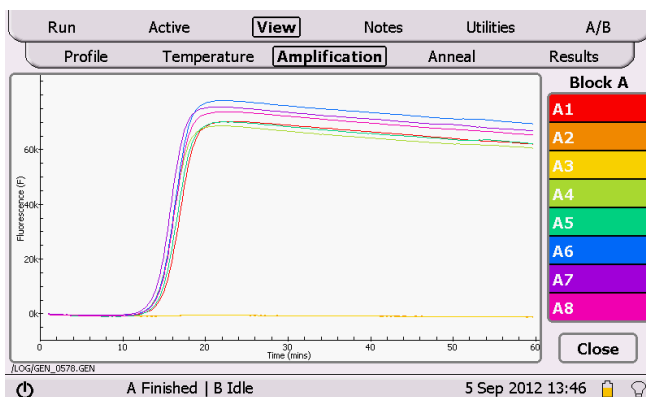
## ADVANCE

Advances to the next phase of the run.



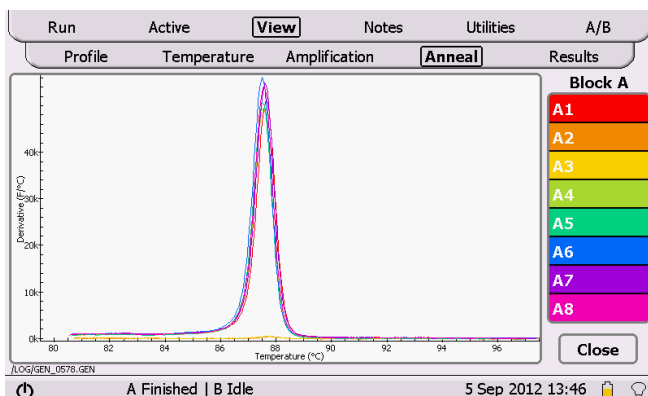
## STOP

The 'Stop' button will stop a run in progress. A confirmation pop up box will prompt 'Yes' or 'No'.



## AMPLIFICATION

This shows the fluorescence data that is being acquired during the amplification phase of the experiment.



## ANNEAL

This shows the fluorescence derivative data that is being acquired during the anneal phase of the experiment.

Well	Amplification mm:ss	Anneal °C
A1	16:30	87.68
A2		
A3		
A4	15:45	87.57
A5	16:15	87.56
A6	16:00	87.55
A7	15:30	87.61
A8	16:00	87.58

## RESULTS

This shows the results of the experiment. The sample name and its respective amplification time and anneal temperature are displayed.

## A/B

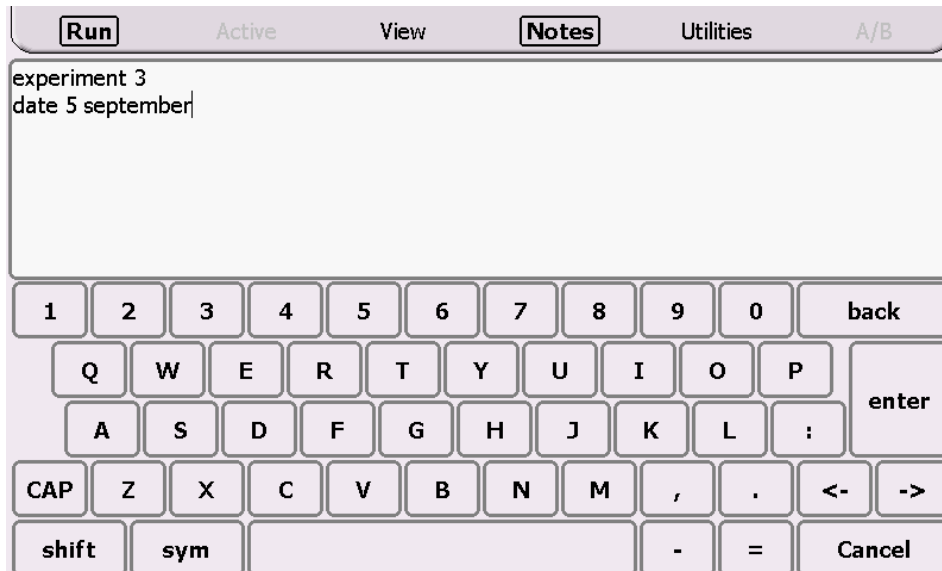
### A/B

Whilst Genie® II is running, the display can switch the views for 'Temperature', 'Amplification', 'Anneal' and 'Results' between block A and block B by pressing the 'A/B' button on the top menu bar. Data for both blocks together is displayed by cycling through the 'A/B' button. When displaying both graphs together, block A data is shown with solid lines, whilst block B data is shown with dashed lines.



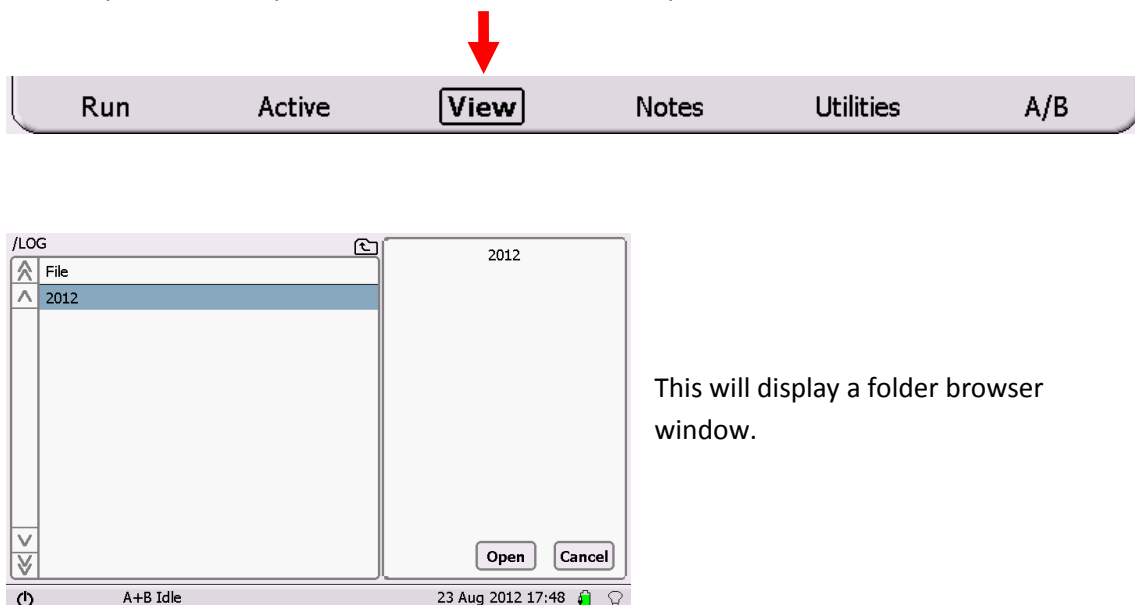
# NOTES

On the 'Notes' screen, notes about the experiment that is being performed can be added and will be stored as part of the log file.

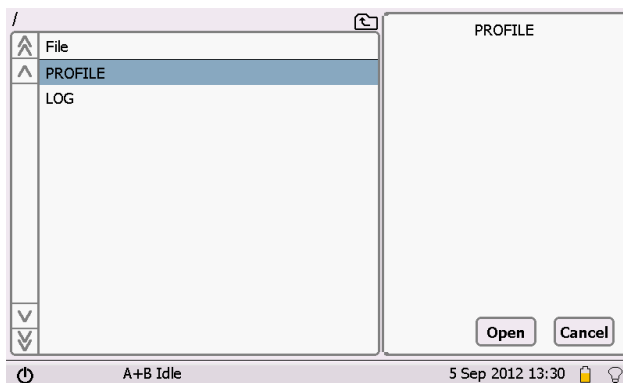


# VIEW

To view previous runs press the 'View' button on the top menu.



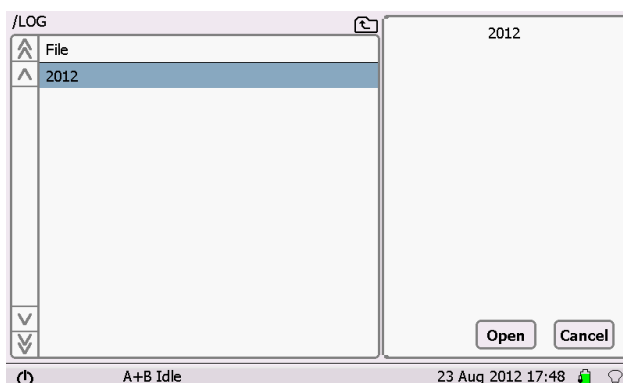
This will display a folder browser window.



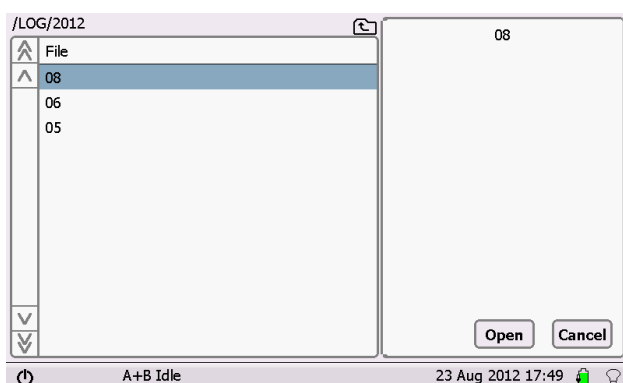
All Genie® II runs are saved in the 'LOG' folder.

Click on 'LOG' then 'Open'.

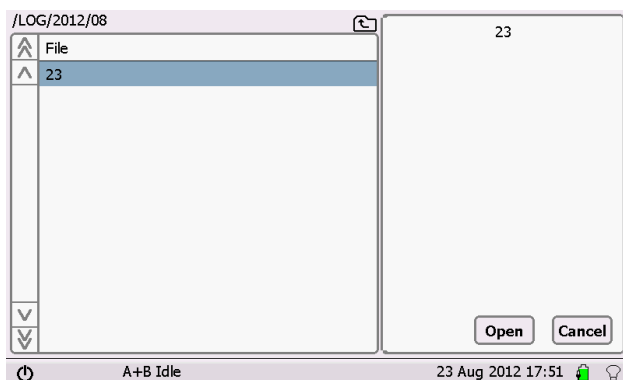
(Alternatively double clicking on the folder name will open the folder)



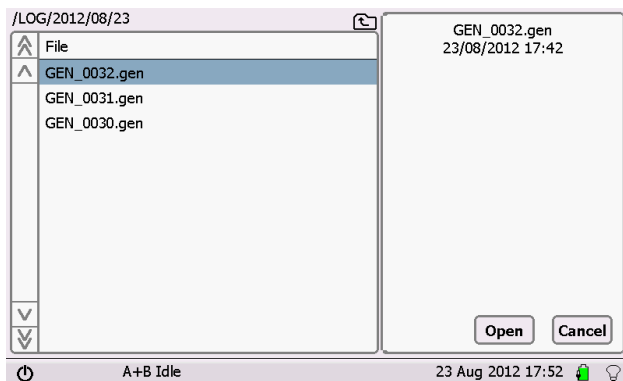
Each run is stored in a folder by date order: **Year**/Month/Day.



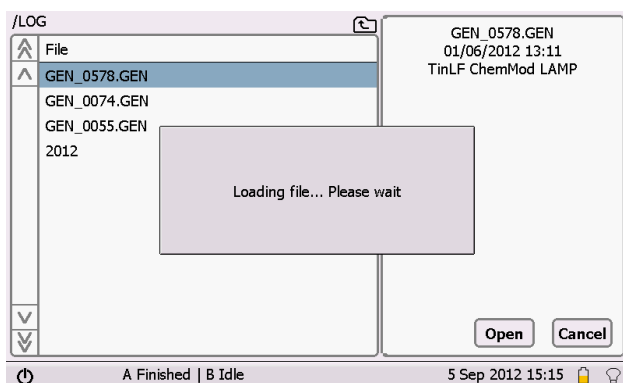
Each run is stored in a folder by date order: Year/**Month**/Day.



Each run is stored in a folder by date order: Year/Month/**Day**.



Each run is then stored numerically in sequential order.

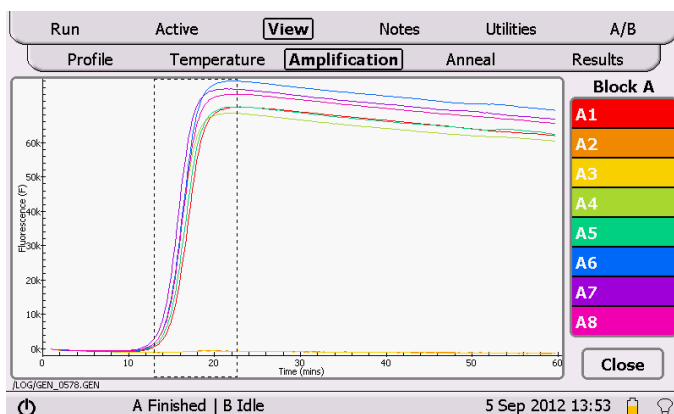


Click on a file, then 'Open' and wait for the file to load.

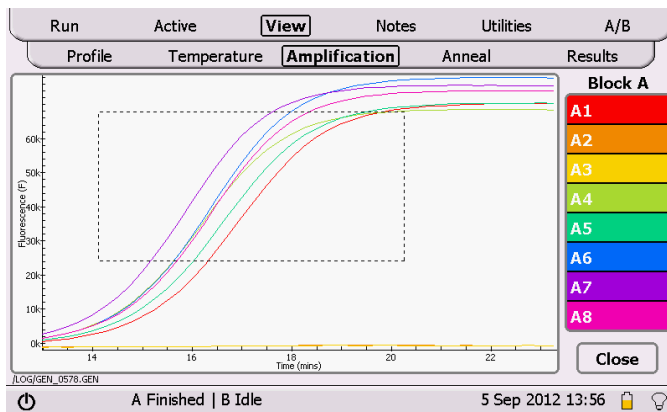
When the file has opened, Genie® II will display the profile that was run, the temperature log, amplification data, anneal data and the results table.

## ZOOMING FUNCTION

Zooming is available on temperature, fluorescence and anneal graphs.



To zoom in on the area of interest, drag to the right and/or down.



To zoom out, drag to the left and/or up.

A double press on the screen will zoom out to the full extent of the graphs.

# Chapter 6

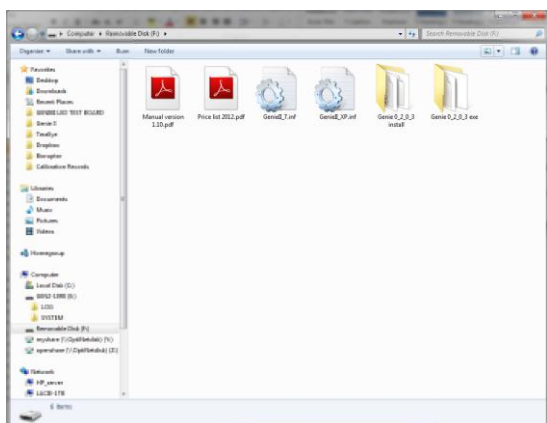
## CONNECTING TO A COMPUTER

Genie® II is a standalone instrument; however, for software updates and other applications, it will need to be connected to a computer.

**IMPORTANT!** Do not plug Genie® II into the computer before installing software.

The PC software can be installed from the USB drive included with Genie® II.

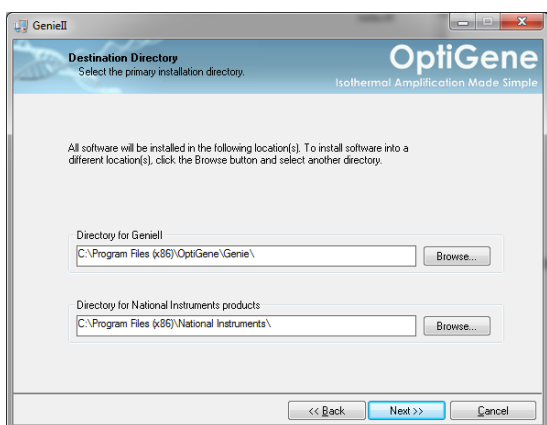
To begin, the Genie® II USB drive should be plugged into a computer.



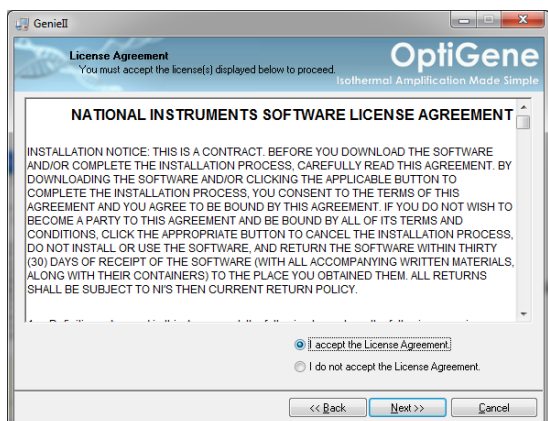
Open the USB drive in 'My Computer'.

Open the folder 'Genie 0\_2\_0\_3 install' and double click 'Setup.exe'.

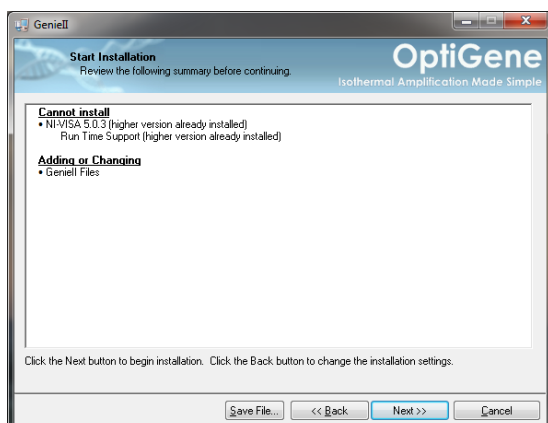
Follow the onscreen instructions.



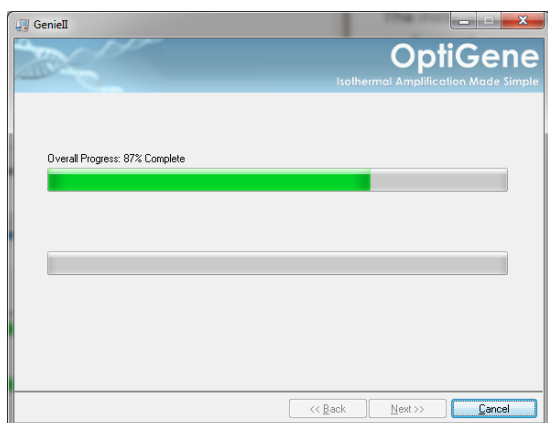
Choose a location for installation to take place.



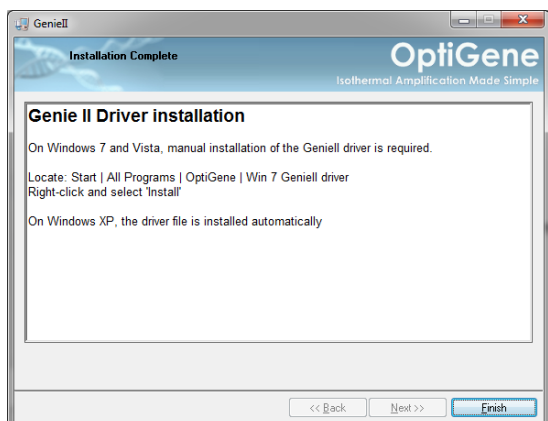
Accept the License Agreement.



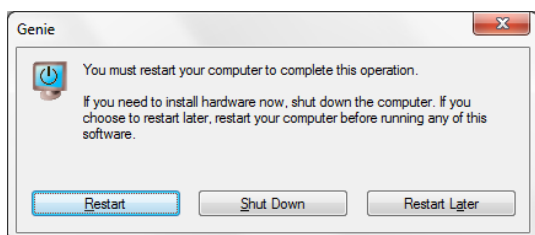
The installer will display the processes being performed.



The installer will copy all necessary files to the computer.

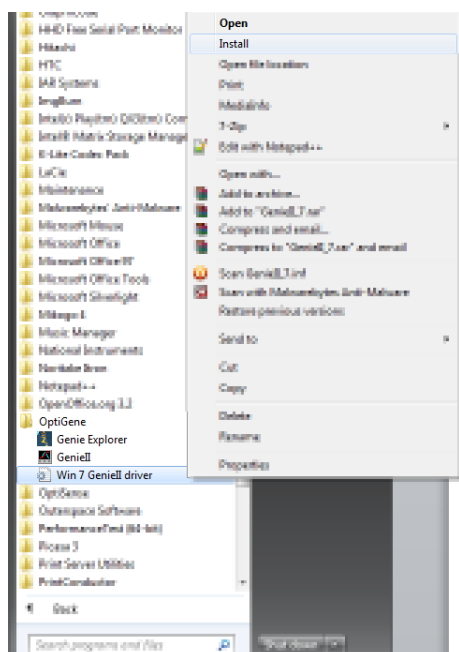


When the installation is complete, it will display information about the device driver installation for Genie® II. To close the window, click 'Finish'.

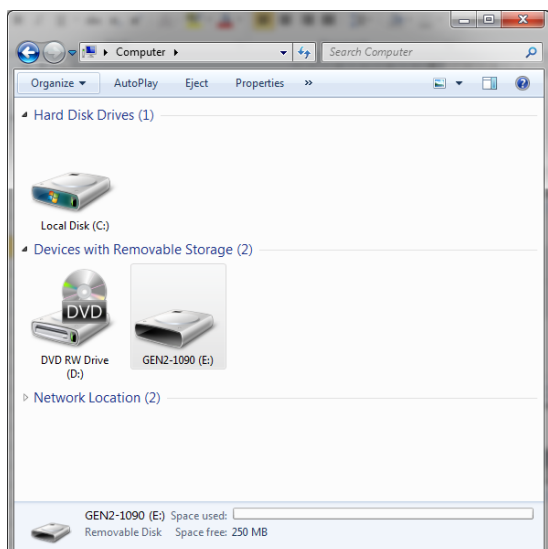


Once the installer has finished, the computer may require a restart to complete the installation process. Close any programs and save any open files before restarting.

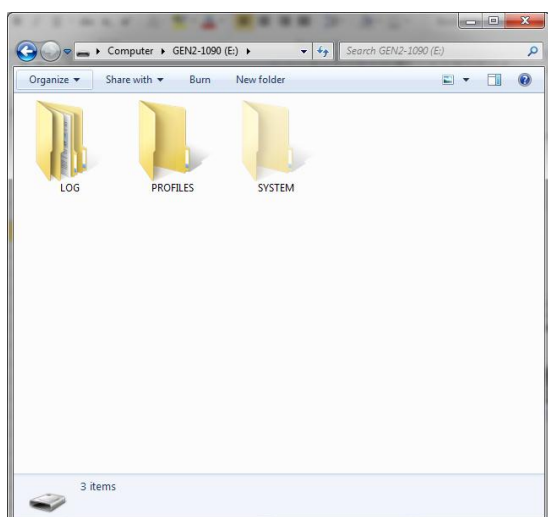
Genie® II can now be connected to the computer. When connected via USB and switched on, Genie® II will appear as a USB device. The driver will automatically install on Windows XP, however on Windows 7 and Vista, manual installation of the Genie® II driver may be required.



This is performed by locating the file. It can be found by clicking *Start > All Programs > OptiGene > "Win 7 GenieII driver"*. Right click on the file and click 'Install'.



Genie® II is now visible as a USB drive in 'My Computer' and the name appears as the instrument's serial number.



Log files and profiles can be copied from Genie® II to the computer and vice versa.

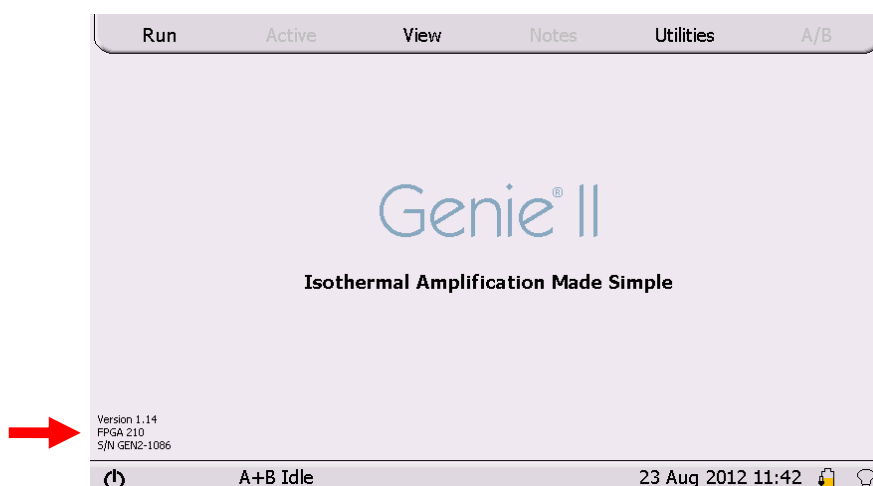
Genie® II is now ready to be used and controlled from the computer.



# GENIE® II SOFTWARE UPDATES

It is recommended to keep the software on Genie® II up-to-date. Upgrading may improve performance and add new features to Genie® II.

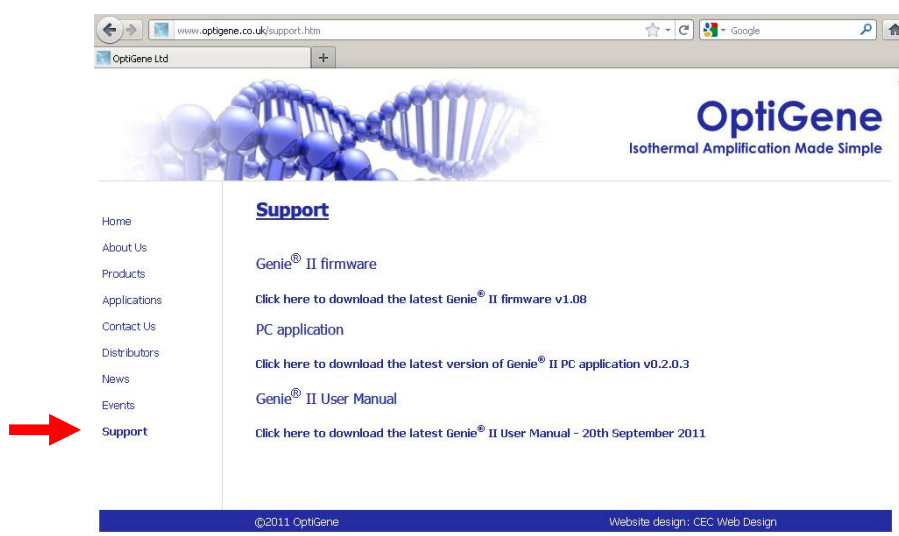
There are two types of software on Genie® II; the firmware, and the FPGA software. The current versions of firmware and FPGA software that are installed on Genie® II are displayed in the bottom left hand corner of the main menu screen.



For Versions 0.99 or earlier please [contact](#) OptiGene Ltd. directly for the correct upgrade files. It is highly recommended to upgrade.

For Versions 1.00 and later go to the OptiGene Ltd. [website](#) and check on the 'Support' page if there is a newer version of firmware available.

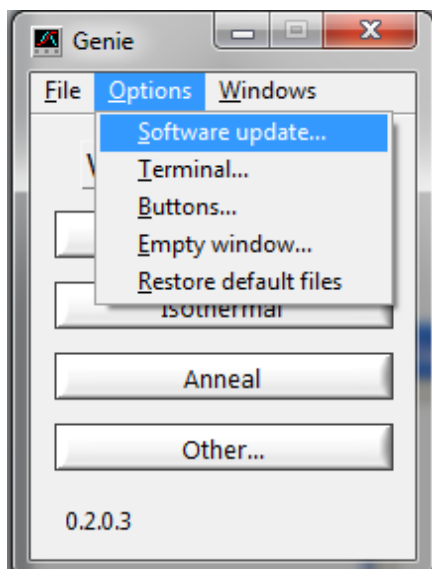
<http://www.optigene.co.uk/support.htm>



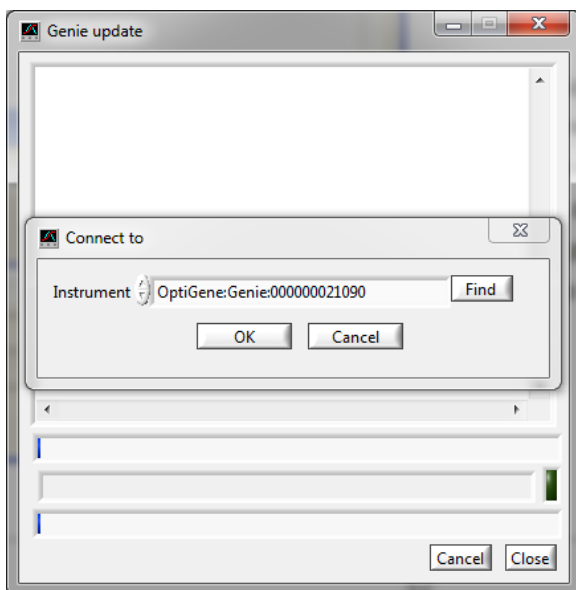
- To download the latest firmware, click on the link and download and save the '.zip' file.
- Open the '.zip' file and extract the contents to a new folder. The contents of the folder will include the latest firmware, FPGA software and this manual.
- If Genie® II already has the latest FPGA software then only the firmware will need to be updated.
- If both firmware and FPGA software updates are required, then update the FPGA software first, followed by the firmware.
- If it is a firmware update, the file will be an '.a20' file, whereas if it is an FPGA software update it will be an '.rbf' file.

## VIA PC

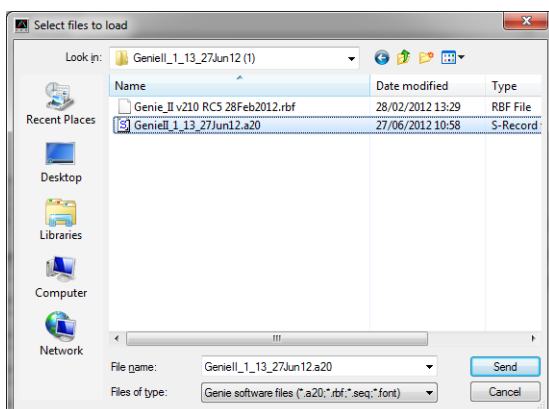
To install the updates, connect Genie® II to a computer, open the Genie® II software and choose 'Software update...' from the 'Options' menu.



Click 'Software update...'.



A pop-up will be displayed. If the instrument is detected, the name of the instrument will be shown. (If it is not shown, check the instrument is turned on and properly connected, and click 'Find' to try again). Click 'OK'



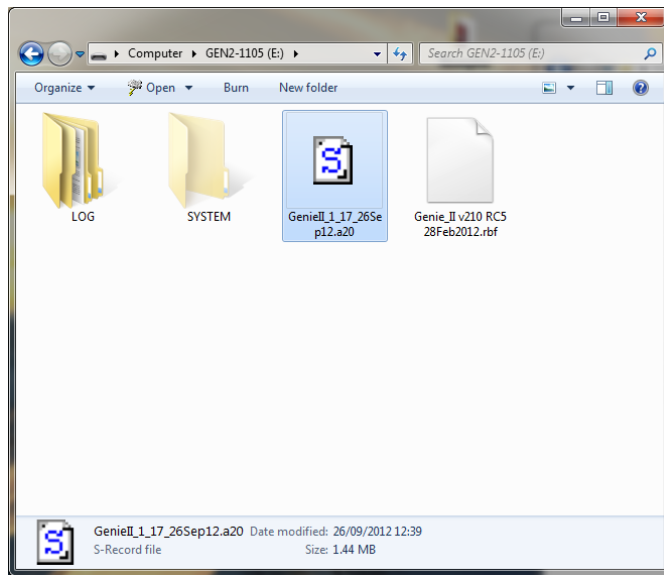
Navigate to the extracted '.zip' file, select the file to upload and click 'Send'.

If it is a firmware update, the file will be an '.a20' file, whereas if it is an FPGA software update it will be an '.rbf' file.

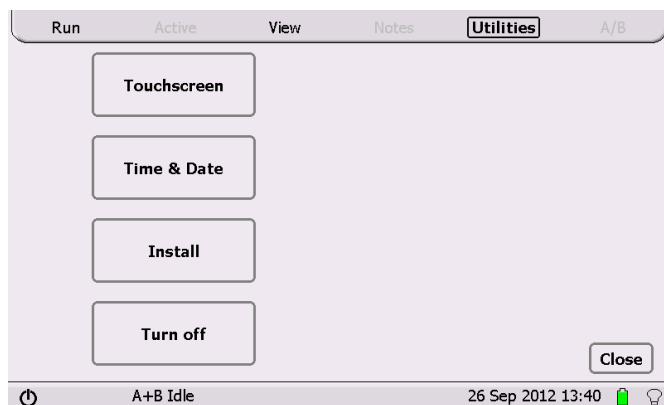
The selected file will be uploaded to Genie® II. If the update was a firmware update, Genie® II will restart when completed. If an FPGA software update was performed, Genie® II will require a manual restart by turning the instrument off and on with the switch on the rear of the unit.

The new software versions will be displayed in the bottom left hand corner of the main menu screen.

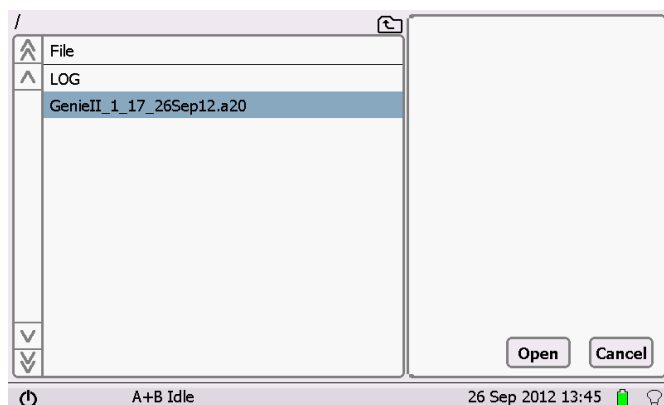
## VIA INSTRUMENT (V1.17 & LATER)



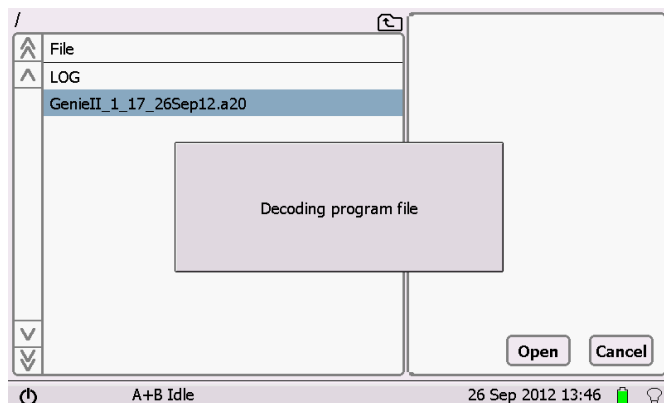
To install the updates, connect Genie® II to a computer. Navigate to 'My Computer' and open the Genie® II drive. The drive is named after the instruments serial number, e.g. GEN2-1105. Copy and paste the firmware or FPGA software files onto Genie® II.



Now on Genie® II click 'Utilities' and click 'Install'. Genie® II will prompt for a file to use for the update.



Click on the file, and click 'Open'. Genie® II will then install the updated software. Please wait for it to finish before trying to do anything else.



The selected file will be uploaded to Genie® II.

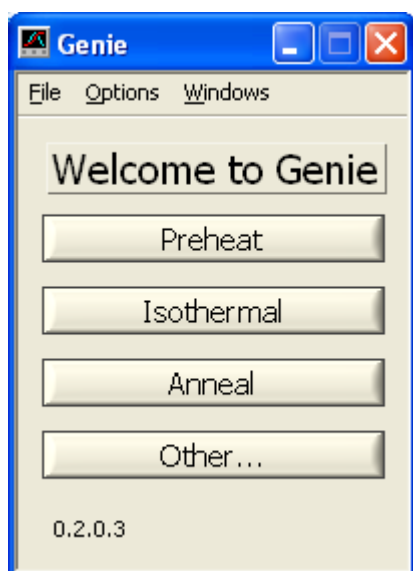
If the update was a firmware update, Genie® II will restart when completed. If an FPGA software update was performed, Genie® II will require a manual restart by turning the instrument off and on with the switch on the rear of the unit.

Genie® II will automatically delete the files when the update has completed.

The new software versions will be displayed in the bottom left hand corner of the main menu screen.

## PC SOFTWARE

### WELCOME SCREEN



From this screen there is the ability to choose to start a new experiment, or review data saved from previous runs.

To carry out an experiment make sure that Genie® II is connected to the computer and switched on.

To start a new isothermal run, click the 'Isothermal' button.

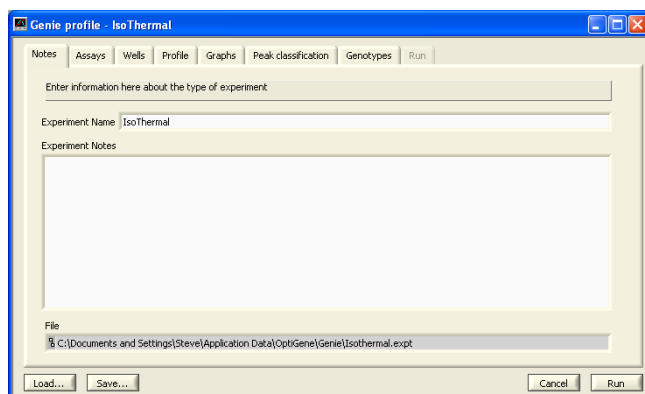
### QUICK START BUTTONS

The Quick Start Buttons are the buttons on the front 'Welcome' screen. Normally they state 'Preheat', 'Isothermal', 'Anneal' and 'Other...' These buttons can be modified very easily, so frequently used experiments or profiles can be stored as a button and be run right from the welcome screen.

- Save the run file then click 'Options' on the welcome screen and click 'Buttons...'.  
• Type in the name for the button and tell the program where the profile file is located.
- The 'Welcome' screen should then be updated with the new button.

# SETTING UP A RUN

## PROFILE SCREEN

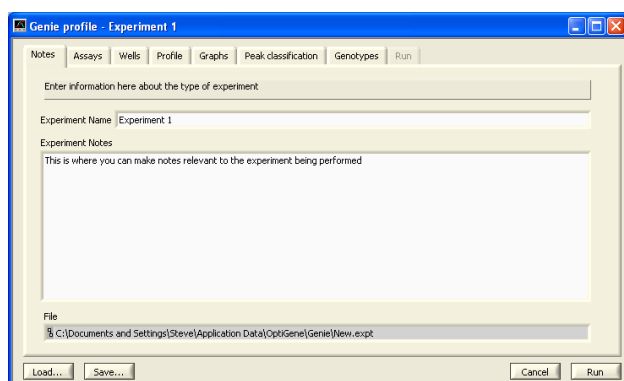


Opening a profile allows modification of all of the experiment parameters:

- Notes
- Assays
- Wells
- Profile details
- Graphs
- Peaks Classification
- Genotypes

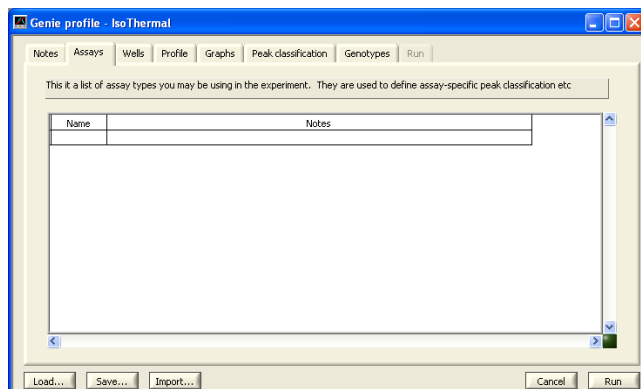
Click upon the desired tab to view or modify settings. It is recommended that users work through the tabs in order, i.e. 'Notes', 'Assays', 'Wells' and 'Profile', in order to add all necessary information before starting a run.

## NOTES



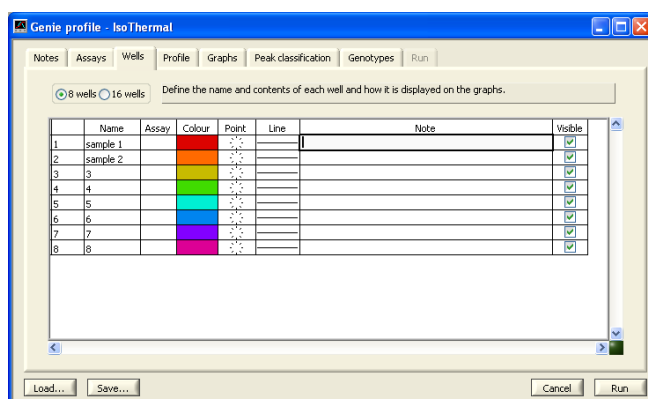
Any experimental notes relevant to the assay being run can be noted here.

## ASSAYS



This is a list of assay types that may be used in the experiment. They are used to define assay-specific peak classification.

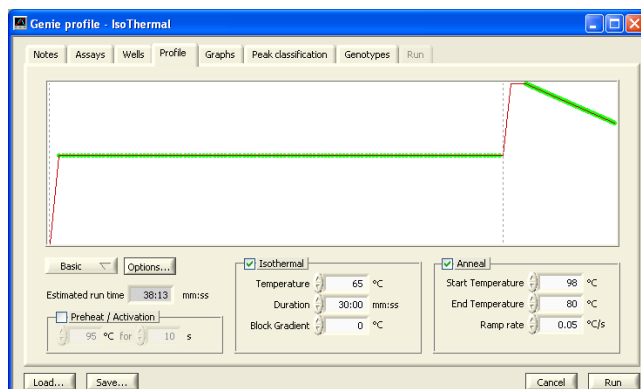
## WELLS



Choose if the experiment is using 8 or 16 wells (default is 8).

There is the ability to enter well names, change plot colours, line styles and choose to make the sample visible on the results display or not.

## PROFILE



The 'Profile' tab allows setting of the thermal profile that is to be used and calculates an estimated run time for the desired profile.



☒ Preheat / Activation

95 °C for 10 s

If activation is required for the enzyme then tick the 'Preheat/Activation' checkbox and set the required temperature and time.

☒ Isothermal

Temperature 65 °C

Duration 30:00 mm:ss

Block Gradient 0 °C

Check the 'Isothermal' checkbox is ticked and then alter the temperature and time to suit the assay conditions.

☒ Anneal

Start Temperature 99 °C

End Temperature 80 °C

Ramp rate 0.1 °C/s

If the assay requires performing an anneal, check the 'Anneal' box and alter the start and end temperature. This dialogue box shows how to set an annealing curve from high to low temperatures.

Note: A melt ' $T_m$ ' peak will be a different temperature from an annealing ' $T_a$ ' peak. This is normal.

## BLOCK GRADIENT

The 'Block Gradient' facility enables a gradient to be set across the blocks. This is especially useful when setting up assays for optimisation of the correct temperature.

☒ Isothermal

Temperature 63.5 °C

Duration 30:00 mm:ss

Block Gradient 7 °C

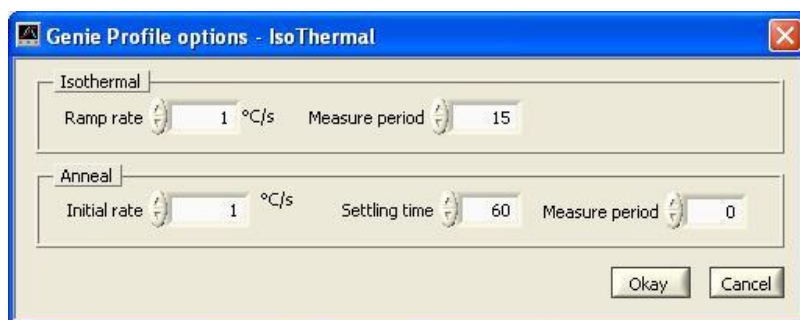
Example of setting up a gradient:

Set centre temperature as 63.5 °C with a gradient of 7 °C.

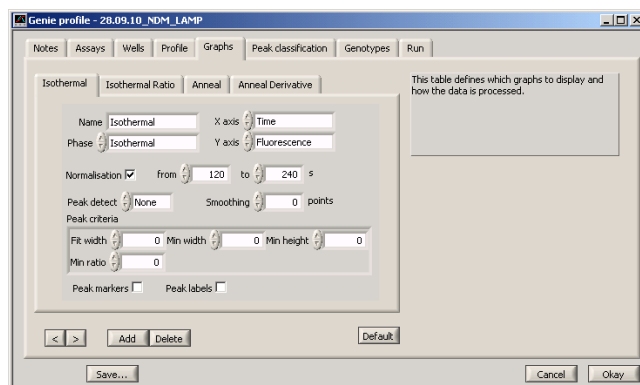
That will set well 1 to 60 °C, well 2 to 61 °C, etc. through to 67 °C in well 8.

## OPTIONS DIALOGUE BOX

The 'Options' button shows a pop-up screen showing several options, which allows changes to be made to the protocol parameters.



## GRAPHS

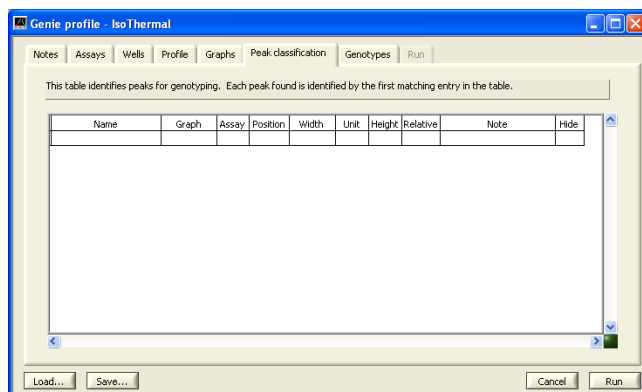


The 'Graphs' tab allows the parameters for different graphs to be adjusted.

Click on the tabs for each graph and alter settings as required.

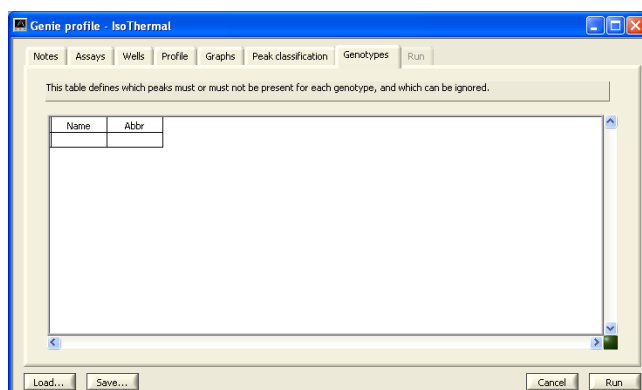
For more information see section  
'Analysis and Graph Options'.

## PEAK CLASSIFICATION



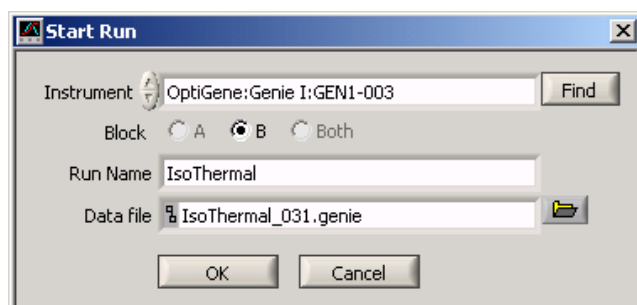
This tab allows identification of peaks for genotyping. Define and enter peaks into the table and the software will automatically label matching peaks.

## GENOTYPE



The 'Genotype' tab allows the user to define peaks, which can be used for automated genotyping by the software.

## RUN DIALOGUE BOX



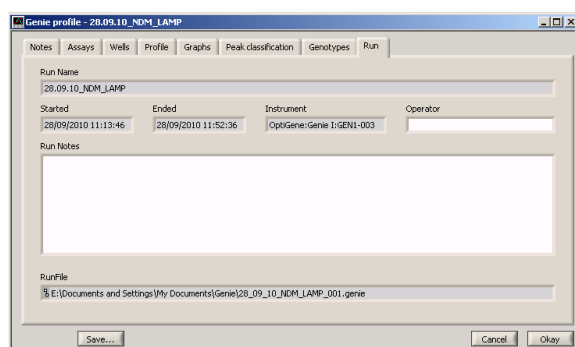
On clicking 'Run' this box will appear. This allows selection of which block is being used and also options for saving of the run file. It is advised to save files in folders/subfolders in the Genie® II directory in 'My Documents'.

Note: If only 8 wells are being used, the option to select 'Both' will be greyed out. In order to use both blocks change the setting in the 'Wells' tab to 16 wells.

Note: Symbols in the 'Run name' will get converted to underscores in the file name.

Note: If a filename is not entered, the software will generate one from the run name and a unique number and save it in the default directory.

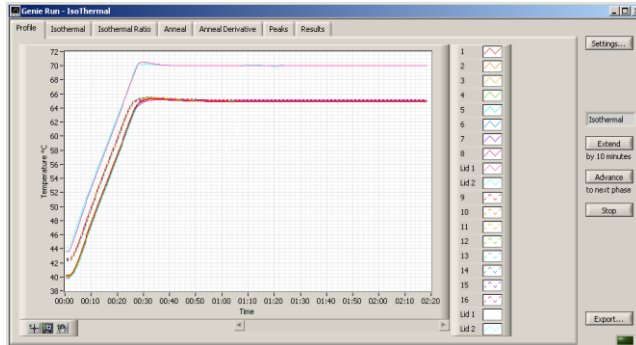
## RUN



The 'Run' tab is an experiment information screen. It displays the profile name, when the profile was run and what instrument it was run on. It allows input for the operator's name, and the ability to add experiment notes.

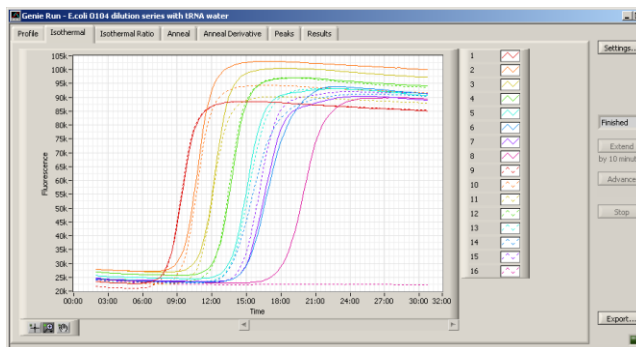
# OBSERVING A RUN

## PROFILE



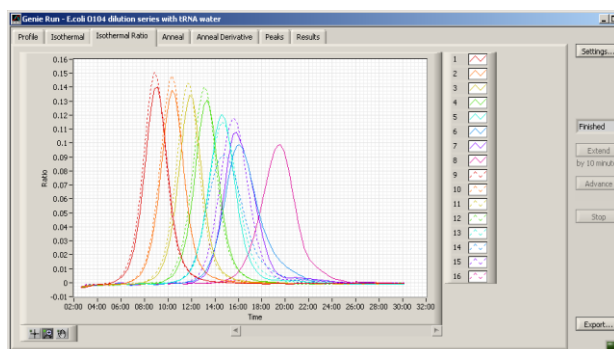
The 'Profile' screen allows observation of the temperatures of the blocks and the heated lids plotted against time. It acts as a thermal record of the run.

## ISOTHERMAL



The 'Isothermal' screen allows observation of the fluorescence of each well plotted against time.

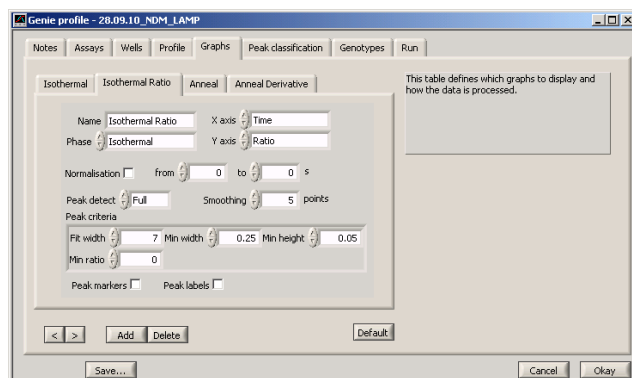
## ISOTHERMAL RATIO



'Isothermal Ratio' shows the amplification take off time in a robust manner.

Isothermal Ratio peak time is equivalent to the  $C_t$  or  $C_q$  in qPCR assays.

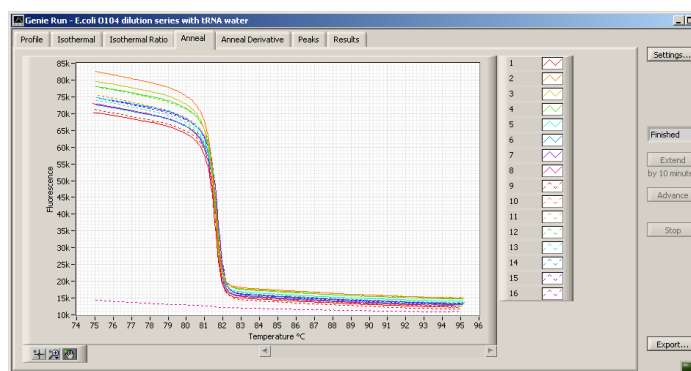
The Genie® II software can detect the peaks and report them automatically. To access the graph options, click 'Settings' and select the 'Graphs' tab, and select the corresponding graph.



Select the 'Peak detect' option ('None', 'Simple' or 'Full'), check the default Peak criteria and click 'Okay'.

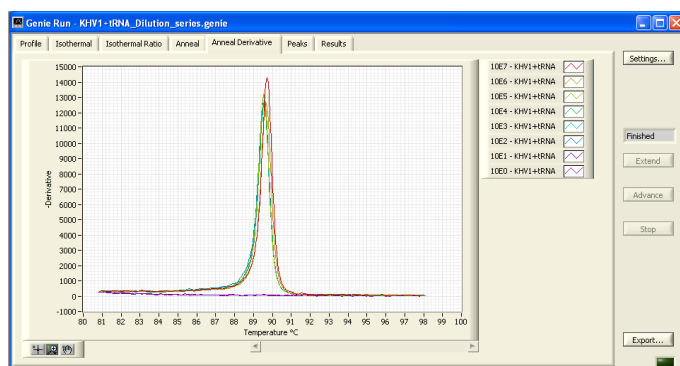
The peak criteria allow the software to display the peaks in the results table.

## ANNEAL

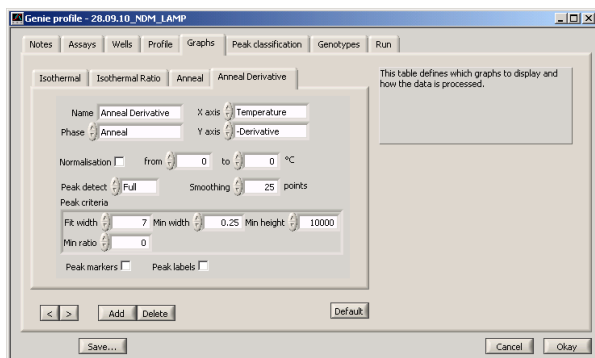


The 'Anneal' tab plots the fluorescence of each well versus temperature in an annealing curve.

## ANNEAL DERIVATIVE



The 'Anneal Derivative' tab shows the derivative fluorescence of each well plotted against temperature, thereby giving the data as a peak.



If the Peak detect box is set on 'Simple' or 'Full' then the peaks will be displayed in the results table.

Check that the peak criteria are correctly set.

## PEAKS

Graph	Well	Name	Peak Position	Value	Width	Height	dV/dx	Relative	Class
Isothermal Ratio	A1	10E7 - KHV1 + RNA	8:13	0.05475	0.30	0.05522	-2.003E-5	1.000	
Isothermal Ratio	A2	10E6 - KHV1 + RNA	9:25	0.05315	0.30	0.05514	-1.916E-5	1.000	
Isothermal Ratio	A3	10E5 - KHV1 + RNA	10:49	0.04727	0.45	0.05011	-1.604E-5	1.000	
Isothermal Ratio	A4	10E4 - KHV1 + RNA	12:19	0.04103	0.44	0.04400	-9.833E-6	1.000	
Isothermal Ratio	A5	10E3 - KHV1 + RNA	15:42	0.02640	1.46	0.02946	-5.549E-6	1.000	
Isothermal Ratio	A6	10E2 - KHV1 + RNA	22:12	0.02183	0.00	0.01725	-9.082E-6	1.000	
Anneal Derivative	A1	10E7 - KHV1 + RNA	89.72	1.429E+4	0.20	1.400E+4	-1.967E+5	1.000	
Anneal Derivative	A2	10E6 - KHV1 + RNA	89.66	1.261E+4	0.20	1.223E+4	-1.723E+5	1.000	
Anneal Derivative	A3	10E5 - KHV1 + RNA	89.55	1.322E+4	0.25	1.293E+4	-1.707E+5	1.000	
Anneal Derivative	A4	10E4 - KHV1 + RNA	89.56	1.267E+4	0.25	1.239E+4	-1.664E+5	1.000	
Anneal Derivative	A5	10E3 - KHV1 + RNA	89.55	1.307E+4	0.25	1.279E+4	-1.661E+5	1.000	
Anneal Derivative	A6	10E2 - KHV1 + RNA	89.55	1.317E+4	0.25	1.288E+4	-1.680E+5	1.000	

This page shows the results in a tabular format and displays the peaks for the different profiles used within the experiment, the position of peaks, the peak width and height.

## RESULTS

Well	Name	Genotype	Abbr.	Peaks
A1	10E7 - KHV1 + RNA	AA BB		8:13 89.72
A2	10E6 - KHV1 + RNA	AA BB		9:25 89.66
A3	10E5 - KHV1 + RNA	AA BB		10:49 89.55
A4	10E4 - KHV1 + RNA	AA BB		12:19 89.56
A5	10E3 - KHV1 + RNA	AA BB		15:42 89.55
A6	10E2 - KHV1 + RNA	AA BB		22:12 89.55
A7	10E1 - KHV1 + RNA	AA BB		
A8	10E0 - KHV1 + RNA	AA BB		

This page shows the results in a tabular format and displays the peaks and genotypes.

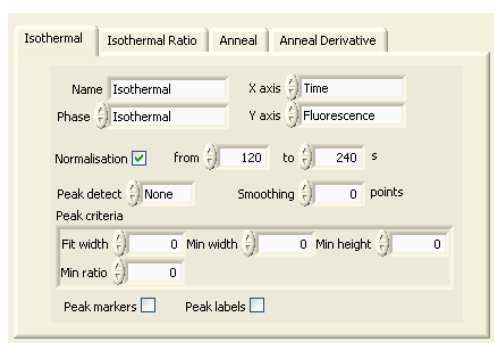
# ANALYSIS AND GRAPH OPTIONS

## GENIE® II GRAPH OPTIONS

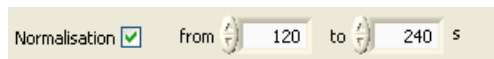
Click the 'Settings' button and it displays the 'Graph Options' window. From this window several aspects of the graphical information can be modified.

There are four tabs on this page and each tab allows the user to check and modify settings appropriate for that tab i.e. 'Isothermal', 'Isothermal Ratio', 'Anneal' & 'Anneal Derivative'.

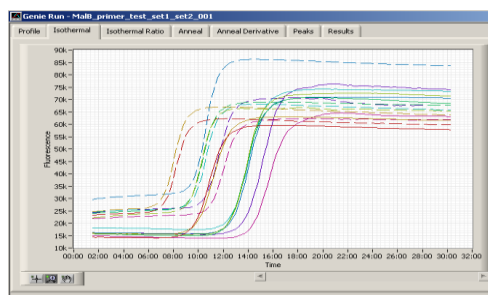
## NORMALISATION



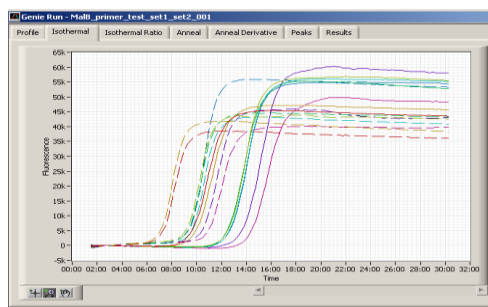
Normalisation allows all the fluorescent signals to be normalised/back grounded over a defined period of time.



Tick the 'Normalisation' checkbox and then set the time for normalisation. E.g. 60 seconds to 240 seconds. Then click 'Okay'.



This screenshot shows data that has not been normalised; the fluorescent signals are different for each well.



With normalisation, the starting points line up and allow for easier analysis.

## PEAK DETECT

The screenshot shows the 'Peak Detect' dialog box with the 'None' option selected. The 'Name' field is 'Anneal Derivative', 'X axis' is 'Temperature', and 'Y axis' is '-Derivative'. 'Normalisation' is unchecked, 'from' is 0, and 'to' is 0 °C. 'Smoothing' is set to 25 points. 'Fit width' is 7, 'Min width' is 0.25, and 'Min height' is 7000. 'Min ratio' is 0. 'Peak markers' and 'Peak labels' are both unchecked.

**None:** no peaks are detected.

The screenshot shows the 'Peak Detect' dialog box with the 'Simple' option selected. All other settings are identical to the 'None' configuration: Name 'Anneal Derivative', X axis 'Temperature', Y axis '-Derivative', Normalisation unchecked, from 0 to 0 °C, Smoothing 25 points, Fit width 7, Min width 0.25, Min height 7000, Min ratio 0, and Peak markers/labels unchecked.

**Simple:** The software looks at the peak and assigns the peak to the highest plotted point. Multiple peaks can be detected.

The screenshot shows the 'Peak Detect' dialog box with the 'Full' option selected. All other settings are identical to the previous configurations: Name 'Anneal Derivative', X axis 'Temperature', Y axis '-Derivative', Normalisation unchecked, from 0 to 0 °C, Smoothing 25 points, Fit width 7, Min width 0.25, Min height 7000, Min ratio 0, and Peak markers/labels unchecked.

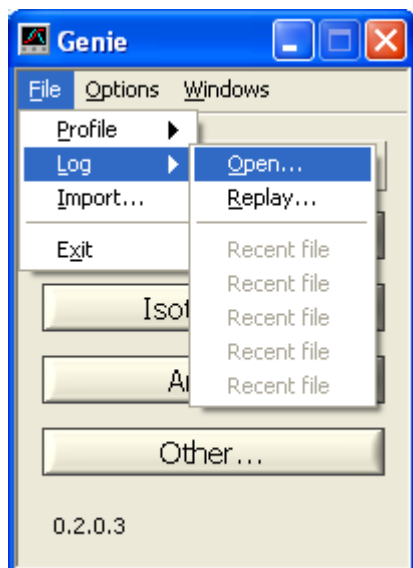
**Full:** The software curve fits and assigns the centre of a peak and can report multiple peaks. It also performs sub-point interpolation.



## OPENING PREVIOUS RUN FILES

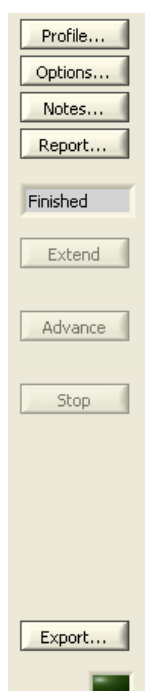
To view previously run data go to the main menu and click 'File' > 'Log' and navigate to the file required.

Files are saved by default in a Genie® II folder in 'My Documents'. The run file is encrypted.



The requested file will be loaded back into the software and analysis of the data can be performed as required.

## ADDITIONAL FEATURES



**Extend:** If more time is required on the current phase press the 'Extend' button and the run will extend by 10 minutes. A box will pop up every time 'Extend' is pressed and the run profile will be updated to show the new time.

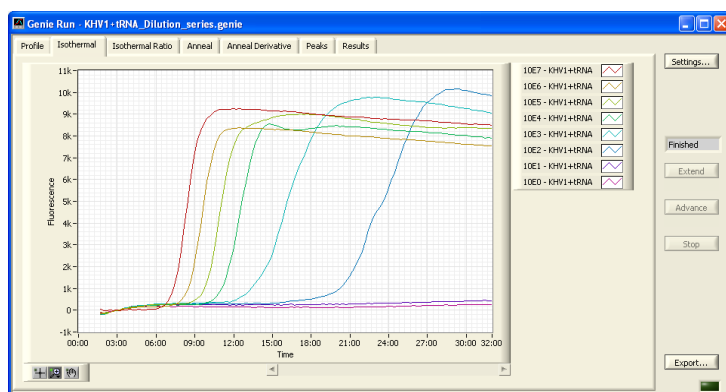
**Advance:** To advance to the next phase, press the 'Advance' button and it will stop the current phase and go onto the next stage specified.

**Stop:** This will stop the run in progress, DO NOT press unless it is required for the run to stop.

**Export:** The results can be exported into a '.txt' file. This allows importing of data into Microsoft Excel or other spread sheet software.

## EXPORT FUNCTION

The Export function allows that data from each graph can be individually exported as a tab-delimited '.txt' file that can be imported into a spread sheet for additional end-user manipulation.



The example below shows if the Isothermal graph is exported, it will export time versus fluorescence for each well.

Time	Fluorescence	Time	Fluorescence	Time	Fluorescence	Time
80.6	-238.3	80.6	-194.8	80.6	-340.2	80.6
95.6	-213.3	95.6	-148.8	95.6	-275.2	95.6
110.6	-160.3	110.6	-91.83	110.6	-198.2	110.6
125.6	-127.3	125.6	-75.83	125.6	-158.2	125.6

The data exported is the data from that graph; if changes are made to the graph for analysis purposes then the graph will need to be re-exported for the changes to be reported.

The following table shows what data is exported for each graph.

<b>Profile Graph</b>	Export time versus temperature for each well as well as the heated lids i.e. 10 temperature readings four times every second throughout the run.
<b>Isothermal graph</b>	Export time versus fluorescence for each well.
<b>Isothermal Ratio graph</b>	Export time versus fluorescence ratio for each well.

Anneal graph	Export temperature versus fluorescence for each well.
Anneal Derivative graph	Export temperature versus fluorescence derivative for each well.
Peaks graph	Export the Well, Name, Peak Position, Value, Width, Weight, $d^2Y/dX^2$ , Relative and Class for each well.
Results graph	Export Well, Name, Genotype, Abbreviation, and Peaks for each well.

# Chapter

# 8

## TECHNICAL SPECIFICATION

Sample number	16 wells (2 x 8 strips)
Sample Volume	10 µl to 200 µl
Touchscreen	TFT / LCD module (800x480) colour, backlit
Heater technology	ceramic substrate with resistive coating
Cooling method	forced convection
Temperature sensor	high-precision thermistor
Temperature control	multi-zone independent digital PID
Temperature range	ambient - 103°C
Temperature accuracy	±0.1°C
Temperature uniformity across block	±0.2°C
Temperature gradient	programmable 1 - 8°C
Optics Source	470 nm LED with high-quality interference filter 40 nm band pass
Detection filter	510 nm longpass photodiode high-quality interference system
Operating temperature	0°C - 40°C
Approvals	CE
Power consumption (maximum)	150W
Dimensions	20cm (H) X 21cm (D) X 30cm (W)
Weight	2kg / 4.4 lb

OptiGene Ltd.  
Unit 5, Blatchford Road,  
Horsham,  
West Sussex,  
RH13 5QR

Tel: +44 (0) 1403-274980

Fax: +44 (0) 1403-271017

[www.optigene.co.uk](http://www.optigene.co.uk)

[info@optigene.co.uk](mailto:info@optigene.co.uk)

Information in this document is subject to change without notice.

OptiGene Ltd. assumes no responsibility for any errors that may appear in this document.

This document is believed to be complete and accurate at the time of publication.

In no event shall OptiGene Ltd. be liable for incidental, multiple, or consequential damages in connection with or arising from use of this document.

Genie® II and its design is a registered trademark of OptiGene Ltd. or its subsidiaries in the UK and other countries.